AMENDMENTS TO THE SPECIFICATION

Please make the following amendments to the specification:

Page 4, paragraph 2, brief description of the figure, lines 11-15

Figures 1A-1~~B~~D show a CLUSTALW alignment of the amino acid sequences of the insect p53 proteins identified from *Drosophila* (Dros_p53, SEQ ID NO:2), *Leptinotarsa* (CPB_p53, SEQ ID NO:4), *Tribolium* (Trib_p53A, SEQ ID NO:6; Trib_p53B, SEQ ID NO:8), and *Heliothis* (Helio_p53, SEQ ID NO:10), with p53 sequences previously identified in human (Human_p53, SEQ ID NO:33), *Xenopus* (Xeno_p53, SEQ ID NO:34), and squid (Squid_p53, SEQ ID NO:35). Identical amino acid residues within the alignment are grouped within solid lines and similar amino acid residues are grouped within dashed lines.

Page 8, last paragraph, bridging pages 8 and 9, page 8 line 25 through page 9 line 4

As used herein, "percent (%) nucleic acid sequence identity" with respect to a subject sequence, or a specified portion of a subject sequence, is defined as the percentage of nucleotides in the candidate derivative nucleic acid sequence identical with the nucleotides in the subject sequence (or specified portion thereof), after aligning the sequences and introducing gaps, if necessary to achieve the maximum percent sequence identity, as generated by the program WU-BLAST-2.0a19 (Altschul *et al.*, J. Mol. Biol. (1997) 215:403-410; ~~http://blast.wustl.edu/blast/README.html;~~ hereinafter referred to generally as "BLAST") with all the search parameters set to default values. The HSP S and HSP S2 parameters are dynamic values and are established by the program itself depending upon the composition of the particular sequence and composition of the particular database against which the sequence of interest is being searched. A percent (%) nucleic acid sequence identity value is determined by the number of matching identical nucleotides divided by the sequence length for which the percent identity is being reported.

Page 24, last paragraph, Lines 22-34

Transposon insertions lying adjacent to a p53 gene can be used to generate deletions of flanking genomic DNA, which if induced in the germline, are stably propagated in subsequent generations. The utility of this technique in generating deletions has been demonstrated and is well-known in the art. One version of the technique using collections of P element transposon induced recessive lethal mutations (P lethals) is particularly suitable for rapid identification of novel, essential genes in *Drosophila* (Cooley *et al.*, Science (1988) 239:1121-1128; Spralding *et al.*, PNAS (1995) 92:0824-10830). Since the sequence of the P elements are known, the genomic sequence flanking each transposon insert is determined either by plasmid rescue (Hamilton *et al.*, PNAS (1991) 88:2731-2735) or by inverse polymerase chain reaction ~~(Rehm, http://www.fruitfly.org/methods/)~~. A more recent version of the transposon insertion technique in male *Drosophila* using P elements is known as P-mediated male recombination (Preston and Engels, Genetics (1996) 144:1611-1638).

Page 26, paragraph 2, lines 8-14

RNAi has also been successfully used in cultured *Drosophila* cells to inhibit expression of targeted proteins (Dixon lab, University of Michigan, ~~http://dixonlab.biochem.med.umich.edu/protocols/RNAiExperiments.html~~). Thus, cell lines in culture can be manipulated using RNAi both to perturb and study the function of p53 pathway components and to validate the efficacy of therapeutic or pesticidal strategies which involve the manipulation of this pathway. A suitable protocol is described in Example 13.

Page 28, first full paragraph, lines 3-19

P elements, or marked P elements, are preferred for the isolation of loss-of-function mutations in *Drosophila* p53 genes because of the precise molecular mapping of these genes, depending on the availability and proximity of preexisting P element insertions for use as a localized transposon source (Hamilton and Zinn, Methods in Cell Biology (1994) 44:81-94; and Wolfner and Goldberg, Methods in Cell Biology (1994) 44:33-80). Typically, modified P elements are used which contain one or more elements that allow detection of animals containing the P element. Most often, marker genes are used that

affect the eye color of *Drosophila*, such as derivatives of the *Drosophila white* or *rosy* genes (Rubin and Spradling, *supra*; and Klemenz *et al.*, Nucleic Acids Res. (1987) 15(10):3947-3959). However, in principle, any gene can be used as a marker that causes a reliable and easily scored phenotypic change in transgenic animals. Various other markers include bacterial plasmid sequences having selectable markers such as ampicillin resistance (Steller and Pirrotta, EMBO. J. (1985) 4:167-171); and *lacZ* sequences fused to a weak general promoter to detect the presence of enhancers with a developmental expression pattern of interest (Bellen *et al.*, Genes Dev. (1989) 3(9):1288-1300). Other examples of marked P elements useful for mutagenesis have been reported (Nucleic Acids Research (1998) 26:85-88~~; and http://flybase.bio.indiana.edu~~).

Page 44 line 31 through page 45, line 14

Analysis of sequences was done as follows: the traces generated by the automated sequencers were base-called using the program "Phred" (Gordon, Genome Res. (1998) 8:195-202), which also assigned quality values to each base. The resulting sequences were trimmed for quality in view of the assigned scores. Vector sequences were also removed. Each sequence was compared to all other fly EST sequences using the BLAST program and a filter to identify regions of near 100% identity. Sequences with potential overlap were then assembled into contigs using the programs "Phrap", "Phred" and "Consed" (Phil Green, University of Washington, Seattle, Washington~~; http://bozeman.mbt.washington.edu/phrap.docs/phrap.html~~). The resulting assemblies were then compared to existing public databases and homology to known proteins was then used to direct translation of the consensus sequence. Where no BLAST homology was available, the statistically most likely translation based on codon and hexanucleotide preference was used. The Pfam (Bateman *et al.*, Nucleic Acids Res. (1999) 27:260-262) and Prosite (Hofmann *et al.*, Nucleic Acids Res. (1999) 27(1):215-219) collections of protein domains were used to identify motifs in the resulting translations. The contig sequences were archived in an Oracle-based relational database (FlyTag™, Exelixis Pharmaceuticals, Inc., South San Francisco, CA).

Page 47 line 27 throughpage 48 line 9

The DMp53 DNA and protein sequences were used to query sequences from *Tribolium, Leptinotarsa, and Heliothis* cDNA libraries using the BLAST computer program, and the results revealed several candidate cDNA clones that might encode p53 related sequences. For each candidate p53 cDNA clone, well-separated, single colonies were streaked on a plate and end-sequenced to verify the clones. Single colonies were picked and the plasmid DNA was purified using Qiagen REAL Preps (Qiagen, Inc., Valencia, CA). Samples were then digested with appropriate enzymes to excise insert from vector and determine size. For example, the vector pOT2, ~~(www.fruitfly.org/EST/pOT2vector.html)~~ can be excised with Xho1/EcoRI; or pBluescript (Stratagene) can be excised with BssH II. Clones were then sequenced using a combination of primer walking and *in vitro* transposon tagging strategies.

For primer walking, primers were designed to the known DNA sequences in the clones, using the Primer-3 software (Steve Rozen, Helen J. Skaletsky (1998) Primer3. ~~Code available at http://www-genome.wi.mit.edu/genome_software/other/primer3.html.~~). These primers were then used in sequencing reactions to extend the sequence until the full sequence of the insert was determined.

Page 59, paragraph 2, lines 8-15

RNAi experiment in tissue culture: RNAi was performed essentially as described previously ~~(http://dixonlab.biochem.med.umich.edu/protocols/RNAiExperiments.html)~~. On day 1, cultures of Drosophila S2 cells were obtained that expressed pMT-HA-DMp53 expression plasmid and either 15 μg of DMp53 dsRNA or no RNA was added to the medium. On the second day, $CuSO_4$was added to final concentrations of either 0, 7, 70 or 700 μM to all cultures. On the fourth day, an alamarBlue (Alamar Biosciences Inc., Sacramento, CA) staining assay was performed to measure the number of live cells in each culture, by measuring fluorescence at 590 nm.

AMENDMENTS TO THE CLAIMS

Please amend the claims to read as follows:

1. (Cancelled)

2. (Cancelled)

3. (Currently amended) The isolated nucleic acid of claim 4 ~~that hybridizes under stringent hybridization conditions with a nucleic acid~~ having a sequence selected from the group consisting of any of SEQ ID Nos:1, 3, 5, 7 , and 9~~, 18, 19, and 21~~.

4. (Currently amended) An isolated nucleic acid molecule that encodes ~~a polypeptide selected from the group consisting of~~ an insect p53 polypeptide, ~~a dominant negative form of said insect p53 polypeptide, a constitutively active form of said insect p53 polypeptide, and a domain of said insect p53 polypeptide selected from the group consisting of an activation domain, a DNA binding domain, a linker domain, an oligomerization domain, and a basic regulatory domain;~~ wherein said insect p53 polypeptide comprises an amino acid sequence selected from the group consisting of: RICSCPKRD (SEQ ID NO:23), KICSCPKRD (SEQ ID NO:24), RVCSCPKRD (SEQ ID NO:25), KVCSCPKRD (SEQ ID NO:26), RICTCPKRD (SEQ ID NO:27), KICTCPKRD (SEQ ID NO:28), RVCTCPKRD (SEQ ID NO:29), KVCTCPKRD (SEQ ID NO:30), FXCKNSC (SEQ ID NO:31), and FXCQNSC (SEQ ID NO:32), wherein X is any amino acid.

5-6. (Cancelled)

7. (Previously amended) The isolated nucleic acid molecule of Claim 4 wherein the insect p53 polypeptide comprises an amino acid sequence selected from the group consisting of any of SEQ ID Nos: 2, 4, 6, 8, and 10.

8-10. (Cancelled).

11. (Previously amended) A vector comprising the nucleic acid molecule of claim 4.

12. (Original) A host cell comprising the vector of claim 11.

13. (Previously amended) A process for producing a p53 polypeptide comprising culturing the host cell of claim 12 under conditions suitable for expression of the p53 polypeptide and recovering the polypeptide.

14-28. (Cancelled).

29. (New) An isolated nucleic acid sequence as set forth in SEQ ID NO:1.

30. (New) An isolated nucleic acid sequence encoding a polypeptide with an amino acid sequence as set forth in SEQ ID NO:2.

REMARKS

Amendments

Specification. Specification has been amended to remove hyperlinks, and also remove issues for objection.

Claims. Claims have been amended to more particularly point out the claimed invention, as further described below. Claim 2 has been cancelled. New claims 29 and 30 are drawn to subject matter considered allowable by the examiner (Paragraph 12 of the Office Action).

No new matter has been introduced as a result of amendments to the specification or the claims. The amendments present the rejected claims in better form for consideration on appeal, and do not introduce any new issues for consideration. Accordingly, entry of the amendment is proper (37 CFR § 1.116).

Election/Restrictions

In paragraph 5 of the Office Action, The examiner states: "It is noted that the original restriction requirement indicated that there was no generic claim. Therefore the nucleic acids are independent and distinct". The examiner also states "The requirement is still deemed proper and is therefore made FINAL".

In the restriction requirement mailed June 20, 2001, the Examiner did not name a generic claim. However, this does not mean that no generic claim existed. At the time, claim 1 was generic, and every claim in the elected group I read upon claim 1. Subsequently, claim 1 was canceled, and dependent claim 4 was amended to be independent and to include the features of claims from group I that were canceled. Currently, claim 4 is the generic claim in Group I, and all the other claims of Group I read on claim 4.

The Examiner's comments about withdrawing "claims containing SEQ ID NO:3-10" from further consideration, and that the "requirement is still deemed proper and is therefore made FINAL" are not understood. First, it is believed that the restriction and species election requirement as set forth in Paper No. 5 was proper, and therefore was not traversed by the applicants. However, it now appears that the present Examiner (who differs from the Examiner who prepared Paper No.5) is, in essence, attempting to impose

a different restriction requirement than that set forth in Paper No. 5, without issuing a new restriction requirement. This is not proper procedure.

The Examiner stated that "no further search will be performed to evaluate SEQ ID NO:3-10 upon determination of allowable subject matter directed to SEQ ID NO:1-2." However, SEQ ID NOs:3-10 are directed to non-elected ***species*** of elected Group 1, and the Examiner's assertion as to how the non-elected ***species*** will be handled is contrary to standard restriction practice (see Sections 803.02 and 806.04 which detail the proper procedure for handling species elections). Further, the original restriction requirement properly stated how claims directed to non-elected species would be handled:

> This application contains claims directed to the following patentably distinct species of the claimed invention:
>
> Applicant must select one of the SEQ ID NOS:2,4,6,8,10,20, 22 **and** one of the SEQ ID NOS: 1,3,5, 18.
>
> ...Upon the allowance of a generic claim, ***applicant will be entitled to consideration of claims to additional species*** which are written in dependent form or otherwise include all the limitations of an allowed generic claim... (Paper No. 5, p. 5; Emphasis added).

Drawings

On paragraph 7 of the Office Action, the drawings are objected to for having holes that overlap the text of the drawings. A formal set of drawings is submitted herewith.

On paragraph 8 of the Office Action, the drawings are objected to for not identifying the sequences by SEQ ID NO in either the drawing or in the brief description of the drawings. The specification has been amended (Page 4, paragraph 2, brief description of the figure, lines 11-15) to identify each sequence in Fig.1 by a SEQ ID NO. Accordingly, a new sequence listing encompassing all SEQ ID Nos is submitted herewith in both paper copy and on disk.

Specification

On paragraph 9 of the Office Action, disclosure is objected to for containing hyperlinks. Amended specification no longer contains hyperlinks.

On paragraph 10 of the Office Action, the title of the invention is stated to not be descriptive of the elected invention. Applicants respectfully disagree. The elected invention is directed to insect p53 tumor suppressor genes and polypeptides, and thus the title of the invention is descriptive. Should the Examiner maintain this objection to the disclosure in the next Office Action, Applicants would appreciate the Examiner's suggestion for an alternative title and/or an explanation as to why the Examiner believes the present title is not adequately descriptive.

Claim Rejections – 35 USC § 112-Description

On paragraph 11 of the Office Action, claims 2-4, 7, and 11-13 were rejected for lack of written description. Applicants respectfully disagree.

Amended claim 4 is drawn to an isolated nucleic acid molecule that encodes an insect p53 polypeptide which comprises an amino acid sequence selected from SEQ ID NO:23 through SEQ ID NO:32. AT this point, nucleic acids encoding SEQ ID NO:28 and SEQ ID NO:32 are under examination.

MPEP 2163 provides:

> "To satisfy the written description requirement, a patent specification must describe the claimed invention in sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention." Further, "Possession may be shown in a variety of ways including description of an actual reduction to practice, or by showing that the invention was "ready for patenting" such as by the disclosure of drawings or structural chemical formulas that show that the invention was complete, or by describing distinguishing identifying characteristics sufficient to show that the applicant was in possession of the claimed invention." Still further, "There is a strong presumption that an adequate written description of the claimed invention is present when the application is filed."

Applicants maintain that they were the first to identify p53 sequences in insect species, and further maintain that their invention is insect p53 sequences, and not just the elected species of Drosophila p53 sequence. At the time of filing of the invention, no insect p53 sequences had been identified, nor was it known whether the p53 tumor suppressor gene existed in insects (Page 2, line 29 to page 3 line 5). The Applicants discovered that insects do have the p53 tumor suppressor gene, and demonstrated the existence of this gene in several insect species. Further, the instant invention provides key conserved

residues that identify an insect p53 sequence. Contrary to examiner's statement "The specification teaches a single Drosophila insect p53 molecule", the specification provides p53 sequences for Drosophila, Colorado potato beetle, Tribolium, and Heliothis - insects spanning 3 different orders of insects and over three hundred million years of evolutionary distance.

The examiner questions the scope of the claim and states: "There is disclosed only a limited number of species, and applicants attempt to claim, on the basis of a single species, any isolated nucleic acid that encodes a polypeptide comprising SEQ ID NO:28 or 32". Contrary to the examiner's statement, Claim 4 is drawn to an isolated nucleic acid molecule that encodes an insect p53 polypeptide comprising SEQ ID NO:28 or 32, and not any isolated nucleic acid that encodes any polypeptide comprising SEQ ID NO:28 or 32. To provide a sampling of the sequences that would contain SEQ ID NO:28 or 32, applicants conducted a sequence homology search for SEQ ID NO:28 and SEQ ID NO:32 using the BLAST program, available at National Center for Biotechnology Information (NCBI).

Exhibit "A" provides the BLAST results for SEQ ID NO:28 against all currently available sequences in public databases. The first matching sequence is Drosophila p53 sequence (GI#21355617 and others). The second matching sequence is also a Drosophila sequence (GI#25009887), which is a longer version of the same p53 sequence. Clustal comparison of the GI#21355617 and GI#25009887 is provided as Exhibit "B". There are no other sequences comprising SEQ ID NO:28 in public databases. Interestingly, the third nearly matching sequence is GI#31207283, which actually matches SEQ ID NO:24 in claim 4, and which is an unannotated sequence for Anopheles gambiae, an insect commonly known as the mosquito. This sequence was introduced into the databases in March 2002. A BLAST query into the sequence reveals that GI#31207283 sequence contains a p53 domain (provided as Exhibit "C"), is most similar to itself and to Drosophila p53 (provided as Exhibit "D"), and as such, is the mosquito p53 sequence.

Exhibit "E" provides the BLAST homology search results for SEQ ID NO:32 against all publicly available sequence databases. The first result is a hypothetical C. elegans protein. C. elegans is a worm, not an insect, and thus, outside of the scope of the instant claims. The second and third results are Drosophila p53 and its variant, as

discussed above. The fourth result is the mosquito p53 sequence discussed above. There are no other insect sequences in public databases that comprise SEQ ID NO:32.

For completion, Exhibits F, G, H, I, J, K, L, and M provide results of homology searches conducted for SEQ ID Nos:23, 24, 25, 26, 27, 29, 30, and 31, respectively. In each instance, either there are no sequences comprising the sequences described, or when there are, the sequences are p53 sequences of an insect.

Taken together, the results of BLAST sequence homology searches alone, provide a very compelling evidence that the claimed invention was clear, concise, and described in detail with sufficient identifying characteristics that a skilled artisan would conclude that the applicant was in possession of the claimed invention.

On page 5 of the office action, examiner states: "Claim 4 broadly encompasses any nucleic acid molecule that encodes a polypeptide comprising SEQ ID NO: 28 or 32". Applicants respectfully disagree. Claim 4 encompasses a nucleic acid molecule that encodes an insect p53 polypeptide comprising SEQ ID NO: 28 or 32, not any nucleic acid molecule. Further, as demonstrated above, the only insect sequences, to date, that comprise the claimed sequences, are insect p53 sequences. The examiner also states "However, there is no known or disclosed correlation between a function and the structure of the claimed invention". The entire application revolves around p53 sequences. Structure and function of known p53 sequences are well-known in the art. All the art-known p53 sequences, including: mammalian and invertebrate p53 sequences which were known prior to filing of the instant application (pages 1 and 2 of the instant specification); the disclosed Drosphila p53 sequence of the instant application (Example 12, page 55 Line 30 through page 58 line 19); and even worm p53 sequence, identified after the filing of the instant application (Derry WB et al (2001) Science 294(5542):591-5. Epub 2001 Sep 13., provided as Exhibit N), function in the cell cycle, regulating DNA-damage induced apoptosis. There is no reason to believe any other identified insect p53 sequences would not share the same function.

MPEP2163 II.A provides:

> "The examiner has the initial burden, after a thorough reading and evaluation of the content of the application, of presenting evidence or reasons why a person skilled in the art would not recognize that the written description of the invention provides support for the claims. There is a strong presumption that an adequate

written description of the claimed invention is present in the specification as filed."

In summary, the applicants have identified and provided several insect p53 sequences; used the Drosophila p53 sequence to identify p53 sequences from vastly varying species of insects (Example 4 pages 47-48); based on the structure of the sequences and their alignment, provided detailed and concise sequence analysis and taught key conserved sequences that would emphasize any insect p53 sequence (pages 14 through 17); and provided detailed functional analysis on at least one insect (Drosophila) to prove that p53 sequences in insects also function in cell cycle, regulating DNA damage induced apoptosis. To date, all known insect p53 sequences in the art meet the metes and bounds of claim 4. The BLAST sequence homology analyses submitted along with this response provides further evidence that the applicants were in possession of the claimed invention at the time of the invention. Thus, given the preponderance of evidence that the applicants have both described the portion of the invention that was reduced to practice (in form of p53 sequences from 4 insects), and also clearly identified distinguishing characteristics for a skilled artisan to identify other p53 sequences, the examiner has not met her initial burden of presenting evidence or reasons why a person skilled in the art would not recognize that the written description of the invention provides support for the claims.

On page 5 of the Office Action, the examiner states: " Further, Claim 3 is directed to an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions to SEQ ID NO:1." Amended Claim 3 does not address hybridization conditions.

CONCLUSION

It is believed that all the objections and rejections raised by the Examiner have been addressed and that the application is in condition for allowance. The Examiner is encouraged to telephone the undersigned with any questions or comments regarding this response.

Respectfully submitted,

Dated: August 20, 2003

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